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Technological possibilities and limitations of microscopic examinations of H&E-stained mammalian cells

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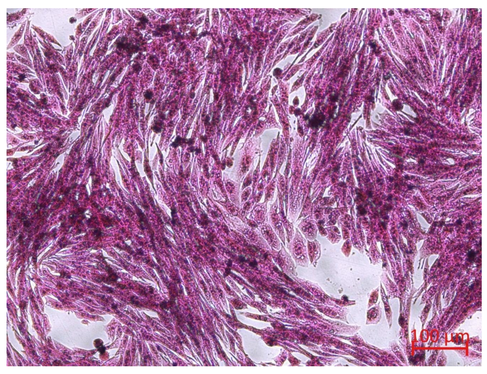
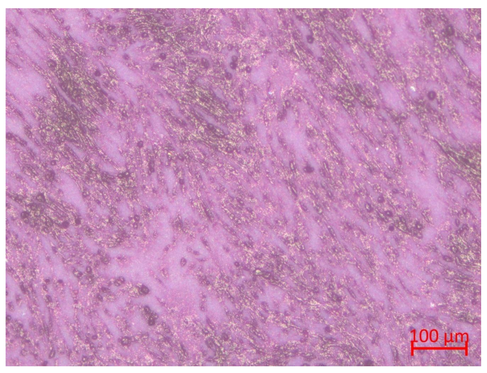
Introduction & Aim

- Microscopic investigation of cell cultures is one of the most commonly used methods in cell biology in order to evaluate cell morphology, growth and structural changes.
- For such examinations, suitable staining methods are often investigated [1-3]. Several dyes, however, have proven problematic for use on cells grown on nanofibrous or 3D-printed biomaterials, as they stain the substrate in a similar way to the cells (left: stained CHO cells on well ground; right: stained CHO cells on polymeric nanofiber mat; from [4]). This suggests optimization of the staining protocol [3].
- An aspect often neglected in such microscopic evaluations is the choice of filters and similar optical modifications of the microscope. The microscopic setup, however, can affect the image quality for diverse fluorescent and other dyes.

→ Here we provide an overview of the influence of diverse microscopic setups.





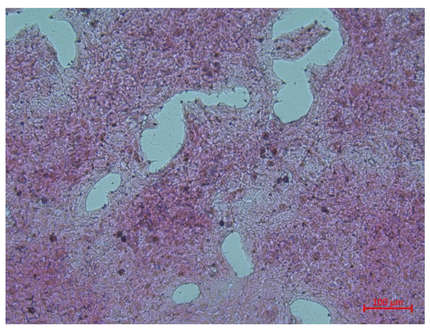
Materials and Methods

- Cells: 3T3-Swiss albino mouse embryonic fibroblasts
- Seeding 6-well plates with 3.5 x 10^5 cells/well in cell culture medium based on Dulbecco's Modified Eagle Medium / Ham's Nutrient Mixture F-12 (DMEM/F-12 medium) and Donor Horse Serum, incubation at 37 °C and 5% CO_2
- Fixation (15 min) with Histofix®, washing with PBS buffer
- H&E staining (while hematoxylin stains basophilic structures such as cell nuclei blue-violet, eosin stains acidophilic parts of the cells pink, especially the cytoplasm)
- Fluorescence microscope Axio Observer 7 (Zeiss) used for cell examination in transmitted light, comparing brightfield images with photographs taken using different phase contrast modes, differential interference contrasts (DIC), crossed polarizers, etc.
- Additional comparison of different exposure times, condenser positions, and white balances

Possible problems in cell growth and staining

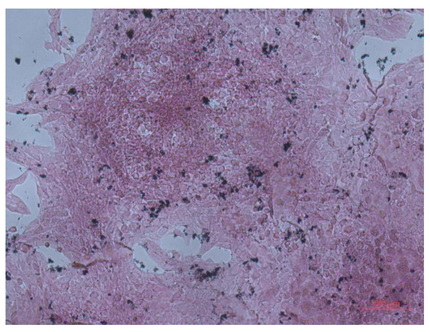
- Starting with highly confluent cell culture can lead to aggregates that are not dissolved during passaging the cells

→ potential changes in cell morphology and adhesion



- Small dark spots after hematoxylin staining – can be attributed to long usage of hematoxylin solution

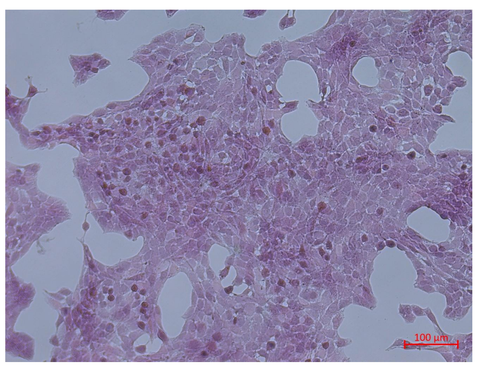
→ filtration before use prohibits this effect



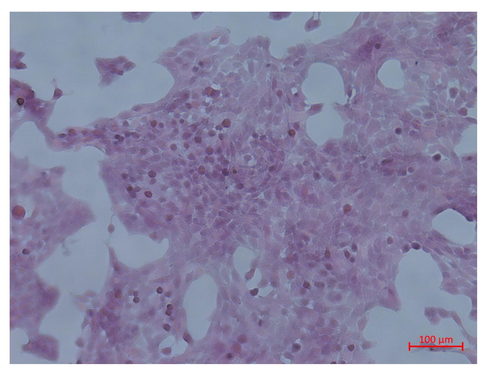
Literature

[1] Wehlage, D.; Blattner, H.; Sabantina, L.; Böttjer, B.; Grothe, T.; Rattenholl, A.; Gudermann, F.; Lütkemeyer, D.; Ehrmann, A. Sterilization of PAN/gelatine nanofibrous mats for cell growth. *Tekstilec* **2019**, *62*, 78-88.
[2] Wehlage, D.; Blattner, H.; Mamun, A.; Kutzli, I.; Diestelhorst, E.; Rattenholl, A.; Gudermann, F.; Lütkemeyer, D.; Ehrmann, A. Cell growth on electrospun nanofiber mats from polyacrylonitrile (PAN) blends. *AIMS Bioengineering* **2020**, *7*, 43-54.
[3] Tanzli, E.; Kozior, T.; Hajnys, J.; Mesicek, J.; Brockhagen, B.; Grothe, T.; Ehrmann, A. Improved cell growth on additively manufactured Ti64 substrates with varying porosity and nanofibrous coating. *Heliyon* **2024**, *10*, e25576.
[4] Dassmann, N.; Brockhagen, B.; Ehrmann, A. Investigation the Optical Contrast Between Nanofiber Mats and Mammalian Cells Dyed with Fluorescent and Other Dyes. *Phys. Sci. Forum* **2024**, *10*, 5.

Results – microscopic settings



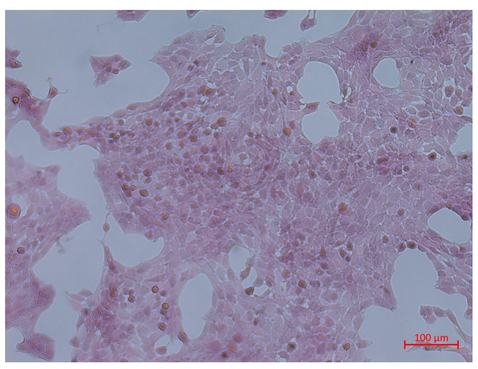
Brightfield image → standard



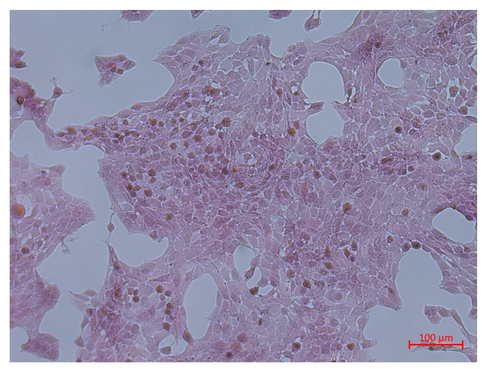
Phase contrast (Ph1) → reduced fine structures

Phase contrast (Ph2) → borders highlighted, fine structures further reduced

Phase contrast (Ph3) → fine structures further reduced



DIC 2 → reduced contrast



DIC 3 → clearer borders

Conclusion and Outlook

- Cell differentiation reduced in Ph1, slightly improved in Ph2
- Crossed linear polarizers do not improve cell differentiation (will be shown in conference paper)
- Correct white balance improves optical quality of micrographs, but does not influence cell differentiation (will be shown in conference paper)
- Optimum condenser setting is necessary to reach the maximum contrast (will be shown in conference paper)
- Optimum light exposure time is necessary to avoid loosing fine structures (will be shown in conference paper)

→ These results show how important it is to carefully select the microscopic setup in order to optimize the quality of images of stained mammalian cells.

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